Author’s response to reviews

Title: Seroepidemiology of infection with Toxoplasma gondii in healthy blood donors of Durango, Mexico

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Author’s response to reviews: see over
Dear Editor,

Attached please find a revised version of our manuscript that had been modified according to the reviewers’ comments. In addition, please find below our response to each of the reviewers’ comments on a point-by-point basis.

We thank very much the valuable comments of the reviewers and we hope the new version of the manuscript may have more success for publication in *BMC Infectious Diseases*.

Kind regards,

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RESPONSE TO THE REVIEWERS’ COMMENTS

Reviewer: Roger Dodd

1. One possible explanation for the low prevalence rates could be that the test itself has low sensitivity. Are there any independent validations of the test that was used?

The sensitivity of the IgG test that is widely used is 94% by the manufacturer. Secondly, we performed quality control of both IgG test and IgM test runs and all test runs were considered valid since they met the insert criteria for this purpose. Thirdly, the low seroprevalence found in this study is comparable with those found in studies of other healthy populations of Durango City where different IgM and IgG detection methods and commercially available brands were used. This information was added to the Discussion section.

2. Why did the authors not test all samples for IgM antibodies? How long may IgM alone be detected prior to the appearance of IgG?

We performed detection of IgM antibodies only in IgG positive samples because the presence of IgM antibodies alone is rarely seen. Anti-\textit{T. gondii} IgG antibodies appears very early after infection (Pfrepper 2005), therefore, the window period between the appearance of IgM and the appearance of IgG is extremely short and the probability to find an IgM positive/IgG negative infected subject seems to be quite low to financially justify a systematic IgM screening in a cross sectional study. In addition, seropositivity to IgM alone is not considered an acceptable diagnostic criterion for acute infection. This information was added to the Discussion section.

3. “Excretes” should be “excreta”.

“Excretes” was replaced by “excreta”.

4. “Logic regression” should be “logistic regression”.

“Logic regression” was replaced by “logistic regression”.

5. Reference 8 and reference 28 appear to be the same article.

Reference 28 was deleted.

6. Table 2: “feaces” should be “feces” (US) or “faeces” (UK).
“feaces” was replaced by “feces”.

7. A quick review of the NLM gateway site reveals that there are a small number of publications that specifically identify Toxoplasma transmission by transfusion (mostly by leukocytes) and, if the authors wish to further reference this point, they may wish to check for these references in addition to numbers 7-10, which mostly deal with transplantation. However, it is acknowledged that transfusion-transmission is not a key component of this particular study.

Since leukocyte transmission of *T. gondii* was not the key component of our study, the authors decided not to include this information.

8. Quality of written English: Needs some language corrections before being published.

The English was improved; the manuscript was reviewed and corrected by a native English speaking teacher.

**Reviewer: Eskild Petersen**

1. *It is major limitation of the study that only 32 persons were seropositive (7.4%) which results in an under power study that may overlook significant risk factors. This should be discussed in relation to the risk factors that came out as significant in the bivariate analysis alone. For instance pork meat and turkey meat, is it really risk factors? Would they have come out in the multivariate analysis with a bigger sample size?*

We have added information in the Statistical Analysis of the Methods section to demonstrate that our results are supported by a sufficient statistical power. In order to obtain reliable results on both the seroprevalence of *T. gondii* antibodies and the presence of any association of the variables with the seropositivity to anti-*T. gondii* antibodies, we performed a number of statistical measures. Firstly, we calculated the sample size. This calculation was performed with the aid of the software Epi Info version 3.3.2 and we used a reference seroprevalence of 8.9% (Alvarado-Esquivel et al, 2005) as expected frequency of the factor under study, 10000 as the size of population from which the sample was selected, a worst acceptable result of 12%, and a confidence level of 95%. The result of the calculation was 314 subjects. Therefore, both our sample size of 432 used and the number of positive subjects found in our study fulfill criteria for a valid statistical analysis in a population with a low frequency of infection. Secondly, we performed not only bivariate analysis but also multivariate analysis and adjustment in the odd ratio to improve the interpretation of our results in a low seroprevalence population. Thirdly, we used the Fisher exact test instead of other statistical tests because it is the most suitable test for analysis of low frequencies. All these statistical methods allowed us to analyze completely and stringently our data and obtain reliable results. The statistical strategies used in our study...
have been similarly used in a number of published epidemiological studies. The fact that we explored a higher number of subjects than expected increased the statistical power of our study. In addition, this study has screened a higher number of blood donors for *T. gondii* antibodies than other previous Mexican studies.

We did not mean that variables “pork meat” and “turkey meat” consumption are risk factors. Indeed, they showed a negative association with seropositivity in the bivariate analysis and turn out to be no significant in the multivariate analysis. This finding indicates that consumption of these meats was not relevant in parasite transmission in our blood donors studied. This information was added in the Discussion section.

2. *I wonder if the association with education and having an earth floor is a proxy for other factors, and this could be discussed. It has been shown from Brazil, that socio-economic status is strongly related to seropositivity with a low socioeconomic status having the highest-prevalence. This was found to be due to consumption of untreated surface water. I do not know if this is relevant to Durango City, but at least it should be discussed.*

The finding that infection decreased with educational level deserves further study. No similar results have been reported, therefore, we expect results of additional studies that may agree or challenge our finding. I am afraid that we can not state that water was influencing our result since untreated water consumption or other water variables were not associated with seropositivity in our study. Socio-economic status does not explain our finding since most donors belonged to a low income population. We may speculate that a high education may be linked to better hygienic sanitary practices than may reduce the transmission of the parasite. This information was added to the Discussion section.

3. *Quality of English: Not suitable for publication unless extensively edited.*

The English was improved; the manuscript was reviewed and corrected by a native English speaking teacher.
Reviewer: Veeranoot Nissapatorn

Materials and methods:
Study population:
1. In this section, it is necessary to clearly elaborate all information of the participants such as type of the study, study site, study period and number of study subjects.

In a new subheading called “Study design and study population”, we have added information about the type of the study, study site, study period and number of study subjects as follows:

We performed a cross sectional study (observational, prospective and descriptive survey) in the two largest blood banks of Durango City, Mexico. These blood banks were: 1) the General Hospital Blood Bank of the Mexican Institute of Social Insurance, and 2) the State Center for Blood Transfusion of the Secretary of Health. Two hundred and one blood donors of the first blood bank and 231 donors of the second blood bank attended from August to September 2006 were enrolled consecutively.

2. It would be better to tell a bite more info of public blood bank in the study area.

We added information in the Study design and study population subheading of Material and Methods section about the blood banks as follows:

Blood banks in Durango City are public, attend mostly low income blood donors, do not pay any blood donation, and give donated blood or blood products in a free manner to hospitals.

3. In inclusion criteria, it was not clear that HIV status or other blood-borne diseases in these blood donors were included or not. If not included, then these subjects were “healthy blood donors”.

We added information on the HIV status in the inclusion criteria as follows:

Inclusion criteria for the study subjects were: 1) voluntary blood donors; 2) aged 18 years and older; 3) regardless their human immunodeficiency virus (HIV) serology status; and 3) who accepted to participate in the study.

None of the blood donors enrolled was positive for HIV serology, therefore we used the term “healthy blood donors” as recommended.

4. Ethical aspects should be included in this section.

The ethical aspects are included in the Methods section. This information was moved up close to the Study design and study population data.
Laboratory tests
This part needs more details;
1. Serum samples
   - Procedure

In the Laboratory tests subheading of Methods section we added information about the procedure for obtaining the serum samples as follows:

Serum samples were obtained by centrifugation of fresh whole blood of the donors. The serum samples were transported from the blood banks to the Laboratory of Microbiology of the Faculty of Medicine (Juarez University of Durango State) where the samples were frozen down and kept stored at –20º C until analysed.

2. Detection of IgG and IgM antibodies to Toxoplasma gondii
   - Where to perform these tests
   - Cut off point value
   - Define positive results

In the Laboratory tests subheading of Methods section we added information about the place the tests were performed, the cut off point value and definition of positive results as follows:

Both tests were performed in the Laboratory of Microbiology of the Faculty of Medicine following the instructions of the manufacturer. For both IgG test and IgM test, we calculated the Toxo G index and Toxo M index, respectively. These indexes are calculated for each determination by dividing the mean values of each sample by cut off calibrator mean value. A sample was considered positive for IgG or IgM when a Toxo G index or a Toxo M index was equal or greater than 1.0. A positive IgG test with a negative IgM test in a donor was interpreted as a chronic infection. A positive IgM test with a positive IgG test in a donor was interpreted as probability of recent infection.

3. Statistical analysis
   - SPSS (name of company, city and country).

We have added the name of the company, city and country in the Statistical analysis subheading of Methods section as follows:

(SPSS Inc. Chicago, IL. USA.

-Line 2-3: For comparison…were used.—revise sentence

We have changed the sentence as follows:

We used the Mantel-Haenszel test, and the Fisher exact test (when cells values were less than 5) for comparison of the frequencies among groups.
Measurement used in qualitative and quantitative variables such as +/- SD, median and range.

We have added data on the standard deviation, median and range of age in the Results section as follows:

The mean age of the blood donors was 30.4 +/- 8.8 years (median: 29 years; range: 18 to 60 years).

Specify a statistical significant of p-value (I see that the authors took sausage consumption of p=0.2 as statistical significant—please refer to statistician for avoiding “bias” of the data).

The statistical significant p value in this study was 0.05 or less. This information was added to the Statistical analysis subheading of the Methods section. Sausage consumption (p=0.2) was not considered significant but this variable was included in the multivariate analysis only to allow potential confounding as other variables with a p value equal or lower than 0.2.

Any reason for taking variables with p? 0.2 in bivariate to be included in multivariate analysis.

We considered variables with a p value equal or less than 0.2 obtained in the bivariate analysis for further analysis by logistic regression to allow potential confounding. This information was added to the statistical analysis subheading of the Methods section.

Line 4: association...T. gondii infection.? between the characteristics of the subjects and Toxoplasma seroprevalence/seropositivity.

We have replaced “T. gondii infection” by “T. gondii seropositivity”.

Table 2 and Table 3: if p?0.2 is not applicable for statistical analysis (refer to statistical analysis in materials and methods)—revise.

As a criterion for inclusion of variables in the multivariate analysis, we considered variables with a p value equal or less than 0.2 obtained in the bivariate analysis to allow potential confounding. However, only a p value of 0.05 or less was considered statistically significant.

Discussion

I would suggest the authors to rearrange the content according to its objectives.
We have rearranged the discussion section. We now have commenced discussing results on
the first objective (seroprevalence) followed by discussion of the data of the second
objective (blood donor characteristics associated with seropositivity). Other general data
was moved up to the Background section.

1. Seroprevalence of toxoplasmosis in general population (blood donor) has been poorly
studied in Mexico.
-Any additional suggestions such as surveillance study or Toxoplasma screening program
in blood donation, organ donors, women (unknown serostatus or seronegative) during
child bearing age and immunosuppressed patients.

There is not any surveillance study or Toxoplasma screening program in blood donation,
women in child bearing age or immunosuppressed patients. Since the Discussion section
was rearranged, we moved up this information to the Background section.

2. Risk factors vs Toxoplasma seroprevalence

I would suggest the authors to discuss according to Results such as demographic and then
other risk factors which makes more systematic and informative.

We now discussed firstly the demographic characteristics and then other characteristics in
the Discussion section.

“Cats at home” → it would be better to have few similar or different studies to challenge
this hypothesis.

We have added information of results of other epidemiology studies as recommended.

From 2nd paragraph, line 2-4 → revise sentence

We have changed the sentence and made it clearer as follows:

In addition, we observed that the frequency of seropositivity to T. gondii decreased as
educational level in donors increased.

Line 4-6 → from this study it is very clear that sample size of both genders are too different
therefore the authors should give comments such as further study needs to be carried out,
etc.

We commented in the Discussion section on the differences in the number of males and
females as follows.

The number of female donors in this study was much lower than that of male donors.
Therefore, further studies are needed to elucidate risk factors associated with seropositivity
in female donors. In contrast, since most blood donors in Durango City are male, and we
studied a higher number of males than females, our findings are representative of the
epidemiology in blood donors in Durango City. We added this information in the Discussion section.

*From the last paragraph, line 5-6 → the authors should give suggestion(s) regarding Toxoplasma via blood transfusion or blood products.*

We added information regarding parasite transmission via blood transfusion in the Discussion section as follows:

Anti-*T. gondii*-specific IgM antibodies may persist for prolonged times after infection [..], but they may also be detected early after infection. Therefore, IgM-positive donors may hold a potential for parasite transmission by blood donation if the infection is recent and parasitemia exists.

*Title needs to be modified? Seroepidemiology of infection with Toxoplasma gondii in (healthy?, see attached in Materials and Methods) blood donors of Durango city, Mexico.*

The term “healthy” was added to the title. The title was changed as follows:

Seroepidemiology of infection with *Toxoplasma gondii* in healthy blood donors of Durango, Mexico

*Background:*
*Line 10-21 → if the authors want these references in Discussion therefore I would suggest to remove these sentences (avoid repeating).*

We have deleted those sentences out of the Background section to avoid repeating in the Discussion section.

*Line 19; discontinuation of references (from 14 then 16, 17) → revise*

The references are now ordered, and reference 15 (now 18) was included.

*Line 26; clinical → refer to Materials and Methods.*

The word “clinical” was deleted.

*Materials and Methods*

*Socio-demographic, clinical and behavioral data.*

*Were all data recorded in a standardized questionnaire?*

We used a standardized questionnaire. This information was added to the Methods section.
Line 6-7: clinical data including blood transfusion or transplant history and behavioral data including…

1. Clinical data should be related with toxoplasmosis (At first, when I read the title, I thought that there are clinical cases of toxoplasmosis involved in this study therefore it is misleading to the reader).

The word “Clinical” was deleted to avoid confusion to the reader.

2. It is more appropriate to use the term of contributing or confounding risk factors for behavioral data in which all possible variables can be included from animal contacts…to…blood transfusion and transplant history.

In the new version of the manuscript we used the term of contributing or confounding factors for behavioral data as recommended.

Line 7-12: behavioral data including…eating outside of the home

The authors should have “definition and duration (such as 3 or 6 months prior to this study)” of these variables which make more reliable information for the study.

The variable “eating outside of the home” was considered as positive when a donor ate outside at least once a year. This information was added to the Methods section.

Results

From Text:
Seroprevalence of anti-T. gondii antibodies
-The authors should give more detail of how many positive anti-Toxoplasma IgG and IgM from each of blood bank.

These data was added to the Results section.

Socio-demographic characteristics associated with seropositivity
Line 2: The mean age of the blood donors was 30.4 years → 30.4 +/- SD?

We added the SD value in this sentence.

Line 2-3: All but one blood donors were born in Mexico → revise sentence

We have modified the sentence to make it clearer.

Line 6-7: in the age group of 45-60 years → where?.

We have corrected that sentence as follows:
The highest frequency of infection (11%) was found in the age group of 35-60 years while the lowest frequency (4.3%) in the age group of 25-34 years.

The difference was statistically significant (p=0.02).

Clinical and behavioral factors associated with Toxoplasma seropositivity
Please rewrite this paragraph and clearly separate Table 2 from Table 3.

We have rewritten this paragraph and made it clearer. In addition, we separated Table 2 (bivariate analysis results) from Table 3 (logistic regression results).

I would suggest that these two headings (socio-demographic characteristics associated with seropositivity and Clinical and behavioral factors associated with Toxoplasma seropositivity) should be combined as;
“Socio-demographic characteristics and other possible risk factors associated with Toxoplasma seroprevalence (seropositivity)”. Then, details are in chronological in order according to Table content.

We have changed the subheading as recommended.

“Cats at home” and “cats in the neighborhood” can be combined as “Contact with cats” whereby the authors can define this variable.

We would like to keep results of separated variables (cats at home and cats in the neighborhood) since these practices are quite different. We considered the variable “cats at home” as a very close contact with a cat (in these cases cats are pets and live in the same house with the owner); while we considered “cats in the neighborhood” when cats are not pets of the donors and are not living in the donor’s house (in these cases cats live in a neighboring house).

Table 1:

At the footnote of this Table, the authors should have short sentence of any significant association between demographic profiles and Toxoplasma seroprevalence (since the column for p-value was not shown in this Table). Then, refer this statement in full length of the text in Results.

We have added a sentence as a footnote to indicate which variable showed a statistically significant difference among the groups analyzed. In addition, we referred this statement in the Results section.
Birth place ➔ the authors should be able to figure out that he or she (blood donors) was born in Mexico or not (because this variable has very contrast in subcategories).

This variable was replace by Born in Mexico (Yes and No) in Table 1.

Occupation ➔ Try to make few groups such as laborer, nonlaborer, and other (the authors may find any significant association with Toxoplasma seroprevalence?) State these definitions at the footnote.

We made fewer groups (only two) and classified them as laborer and no laborer. We added information on these definitions at the footnote.

Housing condition index: To my surprise, only 345 out of 432 were recorded in this study ➔ What happened to 87 records.. whether they are homeless or their houses were not fitted to these categories???

Indeed 87 questionnaires had incomplete housing conditions. We clarified in the footnote of Table 2 that analysis was performed with available data of blood donors. Anyway, we compared results of donors with and without the housing data and there was not any difference. Therefore, this lack of information in housing of 87 donors did not affect our results.

Table 2:

<table>
<thead>
<tr>
<th>Variable</th>
<th>Degree of meat cooking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw or undercooked</td>
<td>10</td>
</tr>
<tr>
<td>Well done</td>
<td>423</td>
</tr>
<tr>
<td>433? (Total number is 432)</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion ➔ revise

We have corrected the number. The total number is indeed 432.

References:

According to the format guideline of the journal, the following references are recommended for correction;

Ref. 1, 11, 14, 16, 17, 18, 21, 25, and 27: Toxoplasma gondii ➔ italic

We have written Toxoplasma gondii in italics in the references 1, 11, 14, 16, 17, 18, 21, 25, and 27.
We have checked the Journal reference style in the Instructions for *BMC Infectious diseases* authors, and for an article within a journal it is not required any number in parenthesis.

Ref 4: Parasitic hosts.\textbf{-- bold}

We have written this sentence in bold letters.


We have corrected the format of this reference.

Ref 5: \ldots 126\ldots \textbf{\textit{126(1-2)}}

We have checked the Journal reference style in the Instructions for *BMC Infectious diseases* authors, and for an article within a journal it is not required any number in parenthesis.


We have corrected the format of this reference.

Ref 8-19 should be made as same as Ref 7 format.

We have corrected the format of references 8-19.

Ref 14, 16, 17\ldots \textbf{\textit{revise (15 was missing in Background)}}

Reference 15 (now 18) is located in the Discussion section.
We have checked the Journal reference style in the Instructions for *BMC Infectious diseases* authors, and for an article within a journal it is not required any number in parenthesis.


We have corrected the format of this reference. All author’s names were included.

Ref 25: ...135... →...135(3-4)...
Ref 26: ...35... →...35(1)...
Ref 27: ...45.....→45(2)....

We have checked the Journal reference style in the Instructions for *BMC Infectious diseases* authors, and for an article within a journal it is not required any number in parenthesis.

Ref 28: remove (it is the same as Ref 8)

The repeated reference was deleted.

*It is an option if the authors want to add one more of Table content for seroprevalence of toxoplasmosis.*

*Total number of Toxoplasma seropositive blood donors = 40 (9.3%)*

<table>
<thead>
<tr>
<th>IgG</th>
<th>IgM</th>
<th>IgG+IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

We can not make such a Table because IgM testing was performed only in IgG positive samples. An explanation about our strategy of testing IgM only in IgG positive samples is given in the Discussion section.

*Quality of English: Need some language corrections before being published.*

The English was improved; the manuscript was reviewed and corrected by a native English speaking teacher.